A. Amendments to the Specification

The specification is amended to add paragraphs for the citation of government sponsorship and for the priority claim, which was inadvertently omitted; these new paragraphs are provided below. In addition, the specification alluded to FIGs. 4 and 5A and 5B, which drawings were not included in the filing. The specification is complete without the omitted drawings. Mentions of those drawings in the specification are deleted below. The remaining drawings 6 and 7 are herein renumbered to FIGs 4 and 5, respectively. The following paragraphs are being amended as indicated:

Statement Regarding Federally Sponsored Research or Development

Some of the work disclosed herein was supported by Small Business Technology Research Grant No. 1-R41-AI50367-01 from the National Institute of Allergy and Infectious Diseases. The government has certain rights in this invention.

Cross Reference to Related Applications

This application claims the benefit of U.S. Provisional Application No. 60/396,928, filed July 16, 2003.

[0015] cancel paragraph

[0016] cancel paragraph

[0017] FIG. 4 ~~6~~ shows the results when fractions from gel filtration were evaluated for protein content (continuous tracing), and assessed for an ability to suppress the PHA-stimulated proliferation of T-cells (vertical bars). Suppression of T-cell proliferation was greatest in fractions corresponding to a mass of 40-100 kDa.

[0018] FIG. 5 ~~7~~ shows the peak active fraction of hNT supernatant from gel filtration which underwent isoelectric focusing using a narrow ampholyte range of pH 4-6 (◊). Isoelectric fractions were assessed for protein content at 280 nm (●). Of the twenty fractions collected and evaluated, only isoelectric fraction #10 (IEF-10) significantly suppressed the proliferation of PBMC induced by PHA (vertical slashed bar) ($p<0.01$). Values are mean ± SD.

[0087] PHA activation of T-cell proliferation results in cross-linkage of the T-cell receptor-CD3 (TCR-CD3) complex and is influenced by accessory cell signals. To determine whether hNT supernatant could suppress the direct activation of T-cells by PMA or ionomycin, independent of an accessory cell influence and independent of TCR-CD3 interactions, hNT supernatants were added to PBMC cultures stimulated with 10 ng/ml PMA, or 100 ng/ml ionomycin, or both. ~~As shown in FIG. 5a,~~ hNT supernatant consistently and significantly suppressed the direct activation of T-cell proliferation by PMA, ionomycin, or both by 99 $\pm$ 1% (n=3), each ($p<0.001$).

[0089] Cell cycle analysis revealed that hNT supernatant did not reduce PBMC viability compared to controls (90.8 $\pm$ 1.7%), that hNT supernatant held PHA-stimulated PBMC in a growth arrested, G_0/G_1 phase (97 $\pm$ 2%), and that the proportion of PBMC in either the S phase or G_2/M phase was reduced by as much as 92% to a mean of only 1.5 $\pm$ 0.7% (n=2) ~~(FIG. 4)~~. The proportions of PBMC undergoing apoptosis, necrosis, and the amount of cellular debris in the modeled events were not different regardless of treatment.

[0103] hNT supernatant was concentrated using YM10 ultrafiltration, and the concentrate fractionated using a Sephacryl S-300 HR gel. Each fraction was assessed for protein content, and pools of five fractions were diluted 1:20 and tested for suppressive activity. Pooled fractions were found to suppress T-cell proliferation over a molecular mass range of approximately 30-100 kDA (FIG. 4 ~~6~~). The peak immunosuppressive active fraction had approximately 7.7 μg/ml of total protein.

[0105] We next used the peak active gel filtration fraction for isoelectric focusing. Preliminary broad range isoelectric focusing indicated that the immunosuppressive protein had an isoelectric point of approximately 5. Consequently, we focused the peak active fraction using a narrow ampholyte pH range of 4-6. Of the twenty isoelectric fractions collected, only fraction #10 suppressed either the PHA or PMA/ionomycin-induced proliferation of T-cells more than 70%, each ($p<0.01$) indicating that the hNT immunosuppressive protein had an isoelectric point of 4.8 (FIG~~S~~. 5~~B and 7~~).